ied the contribution of cytokine gene polymorphisms to TRM in 182 consecutive unrelated donor transplants performed in a single center. Haplotypes for TNF and IL-10 genes were constructed using estimated haplotype analysis. We then analysed haplotype associations with transplant outcome, confirming our findings by multifactorial analysis. The TNF-d microsatellite d4 allele was strongly linked to the TNF-a -1031 C and TNF a5 alleles. The TNF d4/-1031C haplotype when present in both donor and recipient was significantly associated with high TRM, 55% (43 - 67%) versus 21% (12 - 30%) when absent from both, p= <0.01. The donor IL-10 haplotype R2- (IL-10G)-G-C-C was associated with increased risk of TRM, 61% (43-79%) compared with 34% (25-43%), p=0.01. The R3- (IL-10G)-G-C-C haplotype associated with decreased risk, 30% (19-41%), compared with 53% (40-66%), p=0.01. This is the first demonstration that TNF-a and IL-10 polymorphisms independently influence TRM after unrelated donor SCT and emphasises the importance of analysing haplotypes rather than single polymorphic loci. We conclude that analysis of cytokine gene haplotypes could be used pre-transplant to identify donors and recipients who carry a high or low risk of TRM.

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MATURATION STAGE OF CORD BLOOD NATURAL KILLER CELLS

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Absence of killer-cell immunoglobulin-like receptor (KIR) triggering in donor NK cells decreases the rate of relapse, rejection and GVHD and improves survival in clinical haplo-identical hematopoietic transplantation. Like immature NK cells in *in vitro* models of NK cells maturation, most murine neonatal NK cells express CD94/NKG2 but not Ly49 inhibitory receptors (homologue of human KIR). The maturation stage of human neonatal NK cells may thus play a role in the outcome of cord blood transplantation. Some immunophenotypic markers are associated with immaturity in human *in vitro* models of NK cell maturation. We measured, in cord blood (n= 12) and adult blood (n=12) human NK cells, the expression of these markers. No significant difference was noted in the percentage of $CD56^{bright}CD16^{dim/-}$ cells, nor in the expression of KIRs, CD16 and CD25 between the two groups. As described for immature NK cells, CD94/NKG2A expression by cord blood NK cells was high (median : 79 % vs. 31 % for adult NK cells). Immature NK cells are known to have fewer cytolytic granules, but 80 % of cord blood NK cells expressed cytoplasmic granzyme B, in contrast with adult blood NK cells (9 %). We also compared CD28 and CD45RO expression in the two groups. As already demonstrated for cord blood T cells, more cord blood NK cells expressed CD28 (65 % vs. 33 % for adult NK cells) and fewer expressed CD45RO (11 % vs. 43 %). This CD28+CD45RO- phenotype is reminiscent of that of naive T cells. Thus, as opposed to mouse neonatal cells, human cord blood NK cells are not phenotypically immature, except for the expression of CD94/NKG2A. The CD28+CD45RO- phenotype may indicate the absence of prior activation. Studies are underway to establish if these phenotypic data translate at the functional level.

IMMUNE RECONSTITUTION

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HUMAN CYTOMEGALOVIRUS INFECTION IMPAIRS IMMUNE PHENOTYPE AND FUNCTION OF IMMATURE AND MATURE MONOCYTE-DERIVED DENDRITIC CELLS THROUGH DISTINCT MECHANISMS

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Human cytomegalovirus (HCMV) infection is associated with immunosuppression. We asked whether HCMV exerted direct effects on dendritic cells (DCs) because of their formative role in T cell immunity. We evaluated immature and mature monocyte-derived dendritic cells (moDCs) for susceptibility to HCMV infection and the attendant effects on differentiation and function. Endothelial cell-propagated HCMV strains infected both immature and mature moDCs, based on immediate early-1 (IE1) Ag expression. Fibroblast-propagated strains infected only mature moDCs. HCMV replicated much more successfully in immature than mature moDCs, with substantial viral particle release and cell lysis. HCMV-infected, IE1+ gated, immature moDCs downregulated CD83, CD86, and class I and II MHC, while expression of CD40 was upregulated. IE1+ immature moDCs proved resistant, however, to maturation with a combination of inflammatory cytokines (IL-1, IL-6, TNFalpha, PGE2). IE1- gated cells from the same infected cultures upregulated all the above epitopes but expressed high levels of the inhibibtory molecule ILT3. UV-inactivated HCMV could not cause comparable effects, indicating a requirement for viral DNA integrity. IE1+ gated mature moDCs proved more resistant to HCMV-induced down regulation of all epitopes except CD83. Both immature and mature infected moDCs exhibited impaired MLR stimulatory activity, and even moDCs with low rates of infection poorly stimulated the MLR. Transfer of virus-free supernatant from infected moDCs to non-infected moDCs inhibited MLR stimulatory activity to the same extent as an active infection. In conclusion, HCMV infection of immature moDCs is distinct from that of mature moDCs. Infection of both populations impairs immune function, however, by 1/ inhibition of maturation and downregulation of MHC and costimulatory molecules; and 2/ the high expression of ILT3 on the bystander immature moDCs and the secretion of soluble factors that inhibit DC immunostimulatory function. These findings have important implications for immune escape by HCMV infection. Further studies are underway to evaluate HCMV infection of resident populations of DCs like Langerhans cells and dermal/interstitial DCs.

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IL-15 ADMINISTRATION AFTER ALLOGENEIC HSCT ENHANCES CD8+T (AND NK/T) CELL RECONSTITUTION THROUGH INCREASED HOMEOSTATIC PROLIFERATION WITHOUT AGGRAVATING GVHD

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Interleukin-15 (IL-15) is a γc cytokine, which plays an important role in the homeostasis of CD8+ T cells and the development and function of natural killer (NK) cells, NK-T cells, and intestinal intraepithelial lymphocytes. We administered IL-15 (2.5 μg /day/mouse) from day 14-21 or 21-28 (in immune reconstitution studies) or from day 0-10 (in GVHD studies) in murine models for allogeneic hematopoietic stem cell transplantation (HSCT) to determine its effects on T cell reconstitution and function, and graft-versus-host-disease. Post-transplant IL-15 had no effect on overall splenic cellularity but significantly increased donor-derived CD8+CD122+ memory-like T cells and NK-T cells when analyzed at day +28 after allo HSCT. This increase in donor CD8 T cells was associated with increased T cell function as determined in a third party MLR, but did not result in auto or anti-host reactivity. We found in experiments with the adoptive transfer of CFSE-labeled donor T cells that IL-15 could stimulate homeostatic proliferation of donor CD8 cells after a syngeneic HSCT and enhance the effects of IL-7 on homeostatic proliferation. IL-15 had a similar stimulatory effect on the homeostatic proliferation of non-alloreactive donor CD8 T cells after an allogeneic BMT. More importantly, IL-15 did not stimulate alloreactive CD4+ or CD8+ T cells and so far we have not found increased GVHD in two different models with CD4 or CD8 induced GVHD. In conclusion, our data suggest that IL-15 can be administered safely after allogeneic HSCT to specifically enhance CD8 T (and NK/T) cell reconstitution and function without exacerbation of GVHD. IL-15 seems to exert this effect primarily through the specific stimulation of the homeostatic proliferation of non-alloreactive donor T and NK/T cells.